Molecular & Cellular Oncology 2:2, e969651; April/May/June 2015; Published with license by Taylor & Francis Group, LLC

Mighty mouse breakthroughs: a Sox2-driven model for squamous cell lung cancer

Anandaroop Mukhopadhyay and Trudy G Oliver*

Department of Oncological Sciences; University of Utah and Huntsman Cancer Institute; Salt Lake City, UT USA

Keywords: lung cancer, mouse models, squamous, Sox2

Abbreviations: CNV, copy number variation; CRISPR, clustered regularly interspaced short palindromic repeats; HLA-A, human leukocyte antigen-A; IKKα, inhibitor of nuclear factor kappa-B kinase subunit α; JAK, Janus kinase; $Kras^{G12D}$, Kirsten rat sarcoma viral oncogene homolog; Lkb1, liver kinase B1; mTOR, mammalian target of rapamycin; NF-Κb, nuclear factor kappa B; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1; PI-3K, phosphoinositide-3-kinase; Pten, phosphatase and tensin homolog; SCC, squamous cell carcinoma; Sox2, sex-determining region Y-box 2; STAT, signal transducer and activator of transcription; TCGA, The Cancer Genome Atlas; Tp63, tumor protein p63.

Squamous lung cancer is a subtype of non-small cell lung cancer with a poor overall prognosis. We have recently generated a mouse model of squamous lung carcinoma by overexpressing Sex-determining region Y-box 2 (Sox2) and deleting liver kinase B1 (Lkb1) using a lentiviral approach. This model recapitulates the human disease in terms of histopathology, biomarker expression, and signaling pathway activation, making it an excellent model for preclinical studies.

Squamous cell carcinoma (SCC) of the lung is the second most common subtype of lung cancer, with a 5-year survival rate of only 15%. The poor prognosis associated with SCC results from ineffective standard of care chemotherapy and a lack of alternative targeted therapies. Targeted therapies that have positive responses in adenocarcinoma, the other major subtype of non-small cell lung cancer (NSCLC), are often inadequate or contraindicated for SCC. There is an urgent need to identify the genes and pathways driving SCC, which should represent new therapeutic targets.

Numerous studies have identified genetic alterations in lung adenocarcinoma, but only recently has lung SCC been extensively explored at the genomic level (Figure 1). In 2012, The Cancer Genome Atlas (TCGA) Research Network sequenced 178 human SCCs.[1] This study identified therapeutically targetable mutations in lung SCC and also highlighted genetic distinctions between SCC and adenocarcinoma. This altered genomic spectrum in SCC may explain why targeted therapies for adenocarcinomas have historically failed in SCC patients.

The TCGA study also revealed that lung SCCs have an exceptionally high mutation rate. This makes it challenging to distinguish "driver" mutations, which represent promising therapeutic targets, from "passenger" mutations, which are simply carried along for the ride. Mouse models of adenocarcinoma and small cell lung cancer have shown that although mouse tumors have fewer genetic alterations than their human counterparts, they exhibit similar gene expression signatures and acquire key genomic changes found in the human disease.[2,3] Mouse models for lung SCC should thus provide a genetic filter to pinpoint key pathways driving the disease.

There has been an explosion of mouse models for lung SCC over the last 7 years. One of the first models generated involved homozygous inactivation of liver kinase B1 (Lkb1) in the context of Kirsten rat sarcoma viral oncogene homolog $(Kras)^{G12D}$-driven lung tumors.[4] This resulted in a mixed spectrum of lung tumor types, including squamous tumors. A second mouse model was generated by a knock-in of kinase dead inhibitor of nuclear factor kappa-B kinase subunit α (IKKα), which led exclusively to squamous tumors.[5] Recently, a conditional genetic model involving simultaneous loss of Lkb1 and Phosphatase and tensin homolog (Pten) was developed.[6] This model exclusively generated squamous lung tumors and is a major step forward in the field.

In our recent work, we used a rapid, reverse-genetic approach to deliver combinations of clinically relevant genetic alterations specifically to the mouse lung using lentiviruses.[7] Bicistronic lentiviruses were delivered specifically to the mouse lung using intranasal inhalation, allowing constitutive expression of 2 genes driven by 2 separate promoters. Using this approach we discovered that expression

*Correspondence to: Trudy G Oliver; Email: trudy.oliver@hci.utah.edu
Submitted: 08/22/2014; Revised: 08/29/2014; Accepted: 08/30/2014
http://dx.doi.org/10.4161/23723548.2014.969651

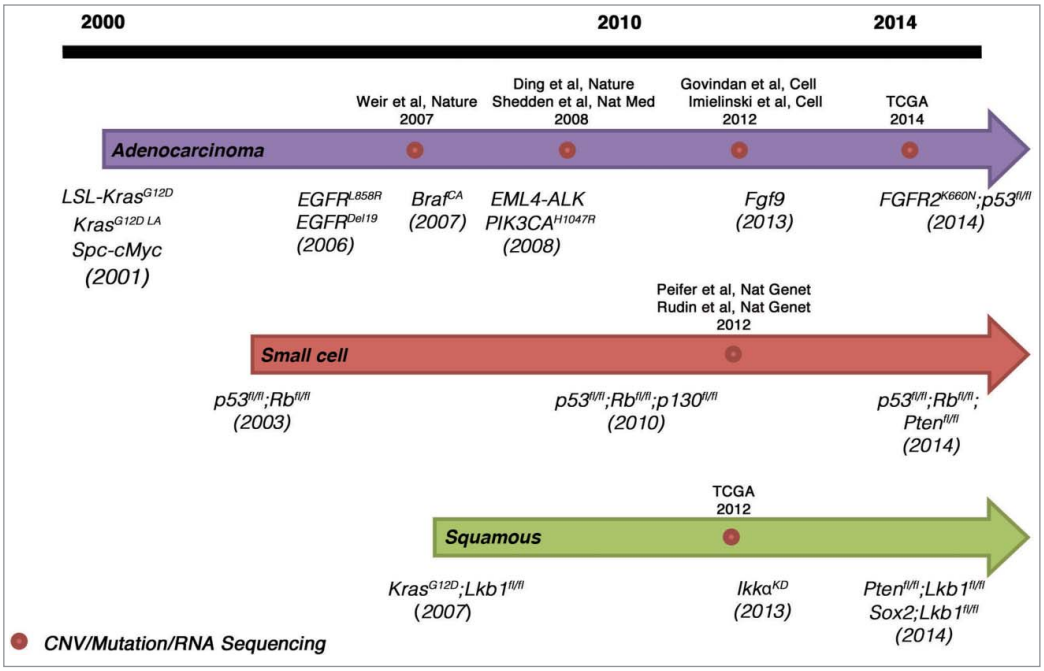

Figure 1. Genetically engineered mouse models of lung cancer. Prominent large-scale genomic analyses and clinically relevant mouse models of lung cancer subtypes are depicted. Mouse models of squamous cell lung cancer have lagged behind those for adenocarcinoma and small cell lung cancer, but a recent explosion of mouse squamous lung tumor models has suggested new therapeutically relevant targets. ALK, anaplastic lymphoma kinase; Braf, v-raf murine sarcoma viral onco homolog B1; CNV, copy number variation; EGFR, epidermal growth factor receptor; EML4, echinoderm microtubule associated protein like 4; Fgf9, fibroblast growth factor 9; FGFR2, fibroblast growth factor receptor 2; IKKα, inhibitor of nuclear factor kappa-B kinase subunit α; Kras, Kirsten rat sarcoma viral oncogene homolog; Lkb, liver kinase B1; c-Myc, avian myelocytomatosis viral oncogene homolog; PIK3CA, phosphatidylinositol 4,5-biphosphate3-kinase catalytic subunit α; p130, retinoblastoma-like 2; p53, tumor protein p53; Pten, phosphatase and tensin homolog; Rb, retinoblastoma protein; Sox2, sex-determining region Y-box2; Spc, surfactant protein C; TCGA, The Cancer Genome Atlas.

of Sox2 together with loss of Lkb1 led to lung SCC with a latency of 5–7 months. These tumors highly resembled human SCC in terms of histopathology and expression of SCC biomarkers including keratin-5, keratin-14, and tumor protein p63 (Tp63).[7] This is clinically important because Sox2 is one of the most commonly overexpressed and amplified genes in lung SCC, but until now has not been demonstrated as a driver of squamous lung tumors.[1,8] Our approach could easily be adapted to test the role of candidate genes or gene combinations that drive lung SCC in a swift and relatively inexpensive manner. With the advent of clustered regularly interspaced short palindromic repeats (CRISPR) technology, this approach is amenable to altering combinations of genes without the time and expense of traditional genetic engineering.

The mouse models highlighted here have identified therapeutically relevant pathways that are active in SCC—phosphoinositide-3-kinase (PtdIns-3K)/mammalian target of rapamycin (mTOR), janus kinase (JAK)/signal transducer and activator of transcription (STAT), nuclear factor kappa B (NF-κB), and programmed death ligand 1 (PD-L1)—thus underscoring the usefulness of *in vivo* models. Squamous tumors generated in both the Pten;Lkb1 loss and Lenti-Sox2;Lkb1 models exhibit activation of the mTOR pathway. Interestingly, immune-related and inflammatory pathways are commonly altered across all mouse models of lung SCC: activated Stat3 pathway in the Lenti-Sox2;Lkb1 mice, activated NF-κB pathway in the kinase dead IKKα knock-in mice, and an increase in immune checkpoint molecule Pdl1 in the Pten; Lkb1 loss mice. These findings are clinically relevant as genomic studies of human SCC reveal somatic alterations in genes involved in the immune response, including human leukocyte antigen-A (HLA-A).[1] In addition, immunomodulatory drugs are exhibiting impressive response rates in clinical trials.[9] Although it is currently not clear how to predict which patients will achieve durable responses to immunotherapies, these mouse models may serve as tools to decipher the mechanisms that dictate therapeutic response.

Advances in mouse models of squamous lung tumors have highlighted many provocative but unanswered questions. It is unclear whether SCCs arise from basal cells or whether the transforming events promote basal cell differentiation. This should be explored by expressing candidate genes that drive lung SCC in specific cell types of the lung. In addition, World Health Organization classification (2004) recognizes 4 variants or subtypes of SCC that can be stratified by gene expression.[10] It is unknown whether the new SCC mouse models resemble these human subtypes. Given that each tumor subtype has different survival outcomes for patients,[10] these subtypes need to be modeled independently to understand how they impact treatment response. Finally, for efficient translation of results obtained from mice to the clinic, it is important to establish paradigms for preclinical testing. Clinically relevant mouse models need to be harnessed to investigate both responsiveness and resistance to targeted therapy.

These are exciting times for lung SCC research. High-throughput genome sequencing has provided a global view of the genetic alterations associated with lung SCC. Functional implications of these changes need to be elucidated using *in vivo* models. Mouse studies have

implicated potential new drug targets for SCC that need to be investigated in the preclinical setting. Models such as these will ultimately impact the lives of patients suffering from this life-threatening disease.

References

1. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012; 489:519-25; PMID:22960745; http://dx.doi.org/10.1038/nature11404
2. Dooley AL, Winslow MM, Chiang DY, Banerji S, Stransky N, Dayton TL, Snyder EL, Senna S, Whittaker CA, Bronson RT, et al. Nuclear factor IB is an oncogene in small cell lung cancer. Genes Dev 2011; 25:1470-5; PMID:21764851; http://dx.doi.org/10.1101/gad.2046711
3. Sweet-Cordero A, Tseng GC, You H, Douglass M, Huey B, Albertson D, Jacks T. Comparison of gene expression and DNA copy number changes in a murine model of lung cancer. Genes Chromosomes Cancer 2006; 45:338-48; PMID:16323170; http://dx.doi.org/10.1002/gcc.20296
4. Ji H, Ramsey MR, Hayes DN, Fan C, McNamara K, Kozlowski P, Torrice C, Wu MC, Shimamura T, Perera SA, et al. LKB1 modulates lung cancer differentiation and metastasis. Nature 2007; 448:807-10; PMID:17676035; http://dx.doi.org/; http://dx.doi.org/10.1038/nature06030
5. Xiao Z, Jiang Q, Willette-Brown J, Xi S, Zhu F, Burkett S, Back T, Song NY, Datla M, Sun Z, et al. The pivotal role of IKKalpha in the development of spontaneous lung squamous cell carcinomas. Cancer Cell 2013; 23:527-40; PMID:23597566; http://dx.doi.org/10.1016/j.ccr.2013.03.009
6. Xu C, Fillmore CM, Koyama S, Wu H, Zhao Y, Chen Z, Herter-Sprie GS, Akbay EA, Tchaicha JH, Altabef A, et al. Loss of Lkb1 and Pten leads to lung squamous cell carcinoma with elevated PD-L1 expression. Cancer Cell 2014; 25:590-604; PMID:24794706; http://dx.doi.org/10.1016/j.ccr.2014.03.033
7. Mukhopadhyay A, Berrett KC, Kc U, Clair PM, Pop SM, Carr SR, Witt BL, Oliver TG. Sox2 cooperates with Lkb1 loss in a mouse model of squamous cell lung cancer. Cell Rep 2014; 8:40-9; PMID:24953650; http://dx.doi.org/10.1016/j.celrep.2014.05.036
8. Lu Y, Futtner C, Rock JR, Xu X, Whitworth W, Hogan BL, Onaitis MW. Evidence that SOX2 overexpression is oncogenic in the lung. PLoS One 2010; 5:e11022; PMID:20548776; http://dx.doi.org/10.1371/journal.pone.0011022
9. Drake CG, Lipson EJ, Brahmer JR. Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. Nat Rev Clin Oncol 2014; 11:24-37; PMID:24247168; http://dx.doi.org/10.1038/nrclinonc.2013.208
10. Wilkerson MD, Yin X, Hoadley KA, Liu Y, Hayward MC, Cabanski CR, Muldrew K, Miller CR, Randell SH, Socinski MA, et al. Lung squamous cell carcinoma mRNA expression subtypes are reproducible, clinically important, and correspond to normal cell types. Clin Cancer Res 2010; 16:4864-75; PMID:20643781; http://dx.doi.org/10.1158/1078-0432.CCR-10-0199